Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.8823-21
Intern Med 61: 2209-2213, 2022
http://internmed.jp

【 CASE REPORT 】

Successful Treatment of Antibody-mediated Pure Red Cell Aplasia Induced by Continuous Erythropoietin Receptor Activator with Prednisolone

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Abstract:
Pure red cell aplasia (PRCA) associated with erythropoiesis-stimulating agents (ESAs), which were first reported in 1998, usually occurs with subcutaneous administration of epoetin alfa (Eprex®). Improvements in ESA storage, handling, and administration methods have reduced the PRCA incidence. Continuous erythropoietin receptor activator (CERA) is a third-generation ESA that is rarely reported to induce PRCA. We herein report a case of CERA-induced PRCA presenting with positive anti-erythropoietin (EPO) and anti-CERA antibodies, which was successfully treated with prednisolone. Clinicians should be aware of the possibility of antibody-mediated PRCA induced by an ESA in CKD patients with anemia with reticulocytopenia and low serum EPO levels.

Key words: antibody-mediated pure red cell aplasia, continuous erythropoietin receptor activator, erythropoiesis-stimulating agents, anti-erythropoietin antibody, anti-CERA antibody

(Intern Med 61: 2209-2213, 2022)
(DOI: 10.2169/internalmedicine.8823-21)

Introduction

Erythropoiesis-stimulating agents (ESAs) are widely used to treat renal anemia in patients with chronic kidney disease (CKD). Antibody-mediated pure red cell aplasia (PRCA) associated with the use of ESAs was first reported in 1998, with several cases reported thereafter (1, 2). Most cases of PRCA occurred in patients who received epoetin alfa (Eprex®) subcutaneously; this method of administration was considered more likely to evoke an immune response than intravenous administration (3). Improvements in the storage and administration of epoetin alfa have resulted in a decrease in the incidence of PRCA in patients with CKD (3).

Continuous erythropoietin receptor activator (CERA) is a third-generation ESA with a methoxy-polyethylene glycol polymer chain integrated into the erythropoietin molecule and a longer elimination half-life than previous ESAs (4). Few cases of PRCA induced by CERA and presenting with anti-epoetin beta pegol antibody have been reported (5, 6).

We herein report a case of PRCA induced by CERA, presenting with both anti-erythropoietin (EPO) and anti-CERA antibodies and successfully treated with prednisolone (PSL).

Case Report

A 69-year-old man with stage 4 CKD due to immunoglobulin A (IgA) nephropathy was started on monthly subcutaneous CERA (Mircera®) as a treatment for renal anemia with a hemoglobin (Hb) level of approximately 10 g/dL. He initially responded well to CERA with a Hb level of approximately 12 g/dL. However, 7 months after the initiation of CERA therapy, his Hb level suddenly decreased to 6.7 g/dL, and the patient fainted when standing up. He was therefore admitted to our hospital. Other notable results were a low reticulocyte count (2,170/μL), normal white blood cell (WBC) count (5,100/μL) with normal differentials, and low platelet count (94×10^3/μL). The platelet count was low at 116×10^3/μL before the initiation of CERA. His serum EPO level decreased to below detection limit (Table 1). Gastroin-

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Received: October 12, 2021; Accepted: November 8, 2021; Advance Publication by J-STAGE: December 28, 2021
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Table 1. Laboratory Data at Admission.

Peripheral blood		Biochemistry		Immunoserology	
WBC	5,100 μL	TP	6.2 g/dL	IgG	889 mg/dL
Stab	5 %	Alb	4.0 g/dL	IgA	177 mg/dL
Seg	53 %	AST	20 IU/L	IgM	46 mg/dL
Lym	27 %	ALT	18 IU/L	C3	70 mg/dL
Mono	7 %	LDH	146 IU/L	C4	26 mg/dL
Eo	8 %	ALP	104 IU/L	CH50	52 U/mL
Baso	0 %	γ-GTP	16 IU/L	ANA	(-)
RBC	217 $\times 10^4$/μL	T-Bil	1.1 mg/dL	DAT	(-)
Hb	6.7 g/dL	D-Bil	0.1 mg/dL	Viral marker	
Ht	19.6 %	Glu	118 mg/dL	Parvovirus B19 IgM (EIA)	0.57
MCV	90.3 fL	UA	8.8 mg/dL	CMV IgM (EIA)	0.18
Ret	0.1 %	BUN	72 mg/dL	CMV IgG (EIA)	9.1
Plt	94 $\times 10^3$/μL	Cr	3.64 mg/dL	EBV VCA IgM (FA)	<×10
		eGFR	14.1 mL/min	EBV VCA IgG (FA)	×160
		CRP	0.01 mg/dL		
		EPO	<0.6 mIU/mL		

WBC: white blood cell, Stab: stab cell, Seg: segmental cell, Lym: lymphocyte, Mono: monocyte, Eo: eosinophil, Baso: basophil, RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, Ret: reticulocytes, Plt: platelet, TP: total protein, Alb: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, γ-GTP: γ-glutamyltransferase, T-Bil: total bilirubin, D-Bil: direct bilirubin, Glu: glucose, UA: uric acid, BUN: blood urea nitrogen, Cr: creatinine, eGFR: estimated glomerular filtration rate, CRP: c-reactive protein, EPO: erythropoietin, IgG: immunoglobulin G, IgA: immunoglobulin A, IgM: immunoglobulin M, C3: complement 3, C4: complement 4, CH50: complement titer (CH50, 50% hemolytic unit of complement), ANA: antinuclear antibodies, DAT: direct antiglobulin test, CMV: cytomegalovirus, EBV: Epstein Barr virus, VCA: viral capsid antigen, EIA: enzyme immunoassay, FA: fluorescent antibody

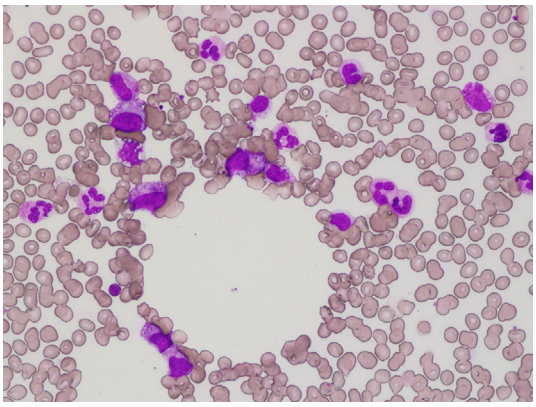

Figure 1. Bone marrow aspiration smear. A bone marrow aspiration smear showed the absence of erythroblasts with normal myeloid cells and megakaryocytes (May-Giemsa staining, 400-fold).

testinal endoscopy revealed no bleeding site, and fecal occult blood test results were negative. A bone marrow aspiration smear revealed the absence of erythroblasts with other normal lineages, consistent with PRCA (Fig. 1). Results of serological tests for parvovirus B19 immunoglobulin M (IgM), cytomegalovirus IgM, Epstein Barr virus capsid antigen IgM, and antinuclear antibodies were negative. Systemic computed tomography (CT) did not reveal a thymoma, malignant lymphoma, or other malignant tumors. Discontinuation of his regular medications (sitagliptin, pitavastatin, lafutidine, irbesartan, febuxostat, and minodronic acid) for three weeks did not improve the anemia. Based on these results, PRCA induced by CERA was suspected.

Subsequently, results from serological studies confirmed the presence of anti-EPO and anti-CERA antibodies [anti-EPO: 1.7110 titer (normal range <0.0815), anti-CERA: 0.0510 titer (normal range <0.0195)]. An examination was performed using an enzyme-linked immunosorbent assay (ELISA) by Chugai Pharmaceutical, Japan. Based on these results, the patient was diagnosed with antibody-mediated PRCA induced by CERA.

The patient's anemic condition did not improve despite discontinuation of CERA on admission; therefore, he was administered immunosuppressive therapy. As the patient had diabetes mellitus, we started treatment with cyclosporine (CyA) rather than corticosteroids (CSs). CyA was started at 100 mg/day and adjusted to maintain a trough concentration between 150 and 250 ng/mL.

However, 3 months after the start of CyA, his reticulocyte count and Hb level did not increase, and he required weekly blood transfusions to maintain a target Hb 7.0 g/dL level. Therefore, the treatment was switched to oral PSL alone at 50 mg per day (1 mg/kg) with insulin therapy. After 24 days, laboratory tests revealed an increased reticulocyte count of 23,900/μL and Hb level of 8.4 g/dL, and he did not require further blood transfusions. The serum EPO level also increased to 68.4 mIU/mL. After 2 weeks of the initial dose, PSL was reduced by 5 mg every week. After reducing the

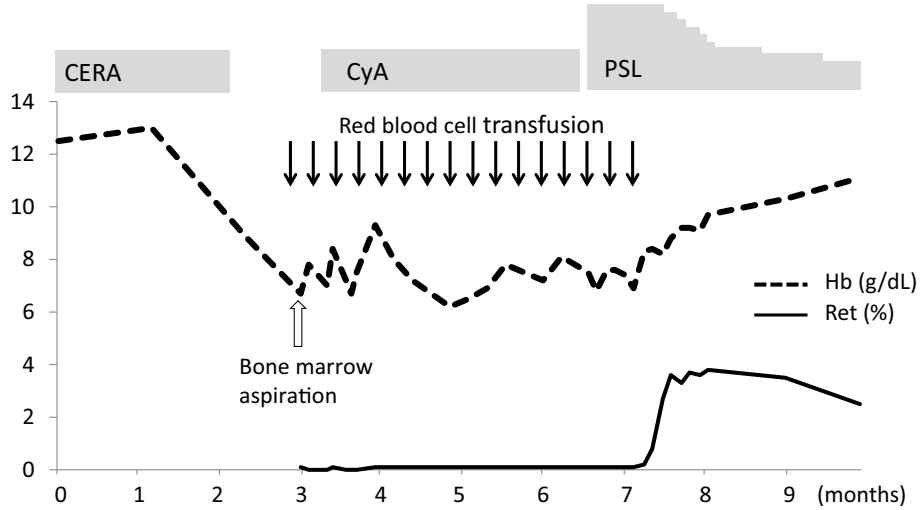

Figure 2. **Patient's clinical course. PRCA did not improve following the discontinuation of CERA and administration of CyA, and weekly blood transfusions were required. After the immunosuppressive agent was changed from CyA to PSL, the anemia improved. CERA: continuous erythropoietin receptor activator, CyA: cyclosporine, Hb: hemoglobin, PRCA: pure red cell aplasia, PSL: prednisolone, Ret: reticulocytes**

dose of PSL to 25 mg, it was reduced by 5 mg every 3 weeks. Three months after the initiation of PSL treatment, levels of anti-EPO and anti-CERA antibodies were undetectable, and his Hb level has remained at approximately 11 g/dL with 5 mg/day PSL (Fig. 2).

Discussion

PRCA is a rare syndrome characterized by severe normochromic normocytic anemia associated with reticulocytopenia and the absence of erythroblasts from otherwise normal bone marrow (7). PRCA is a primary hematologic disorder, but it can also occur secondary to infections (especially parvovirus B19), hematologic malignancies, autoimmune diseases, thymoma, and exposure to drugs and toxic agents (8).

The incidence of PRCA associated with anti-EPO antibodies in CKD patients with anemia treated with ESAs began to increase in 1998 and peaked in 2001 (3). Most of these cases were in patients who were administered epoetin alfa (Eprex®) subcutaneously, and it is thought that polysorbate-80 from uncoated rubber syringe stoppers caused PRCA associated with epoetin alfa (9, 10). In the case of PRCA induced by ESAs, the development of neutralizing anti-EPO antibodies, which recognize all available ESAs as well as endogenous EPO, blocks the interaction between EPO and its receptor (2). The mean interval between initiating ESA therapy and the onset of PRCA was 9 (range, 2-63) months (9).

Recently, the incidence of PRCA induced by ESAs has decreased owing to improvements in the storage of ESAs and in the route of administration (3, 10). CERA, a third-generation ESA approved in 2007, has a methoxy-polyethylene glycol polymer chain integrated into the erythropoietin molecule and a prolonged half-life (approximately 130 hours), allowing for extended dosing intervals (every 2 or 4 weeks) (11). This agent is generally well tolerated, with most adverse events being of mild to moderate severity (11), but there are a few case reports of PRCA related to CERA (5, 6) (Table 2). In addition, darbepoetin alfa, another ESA, is also widely used for renal anemia in patients with CKD, and several cases of antibody-mediated PRCA have been reported (12-16). In almost all cases, ESAs were administered subcutaneously, and immunosuppressive therapy was required (Table 2).

Treatment of antibody-mediated PRCA induced by ESAs generally requires discontinuation of ESAs and administration of immunosuppressive agents such as CyA and CS to suppress anti-EPO antibodies (2). The response rates to CyA and CS in patients with anti-EPO antibody-mediated PRCA were 67% and 56%, respectively (17). In our case, initial CyA therapy did not improve the hematological parameters of PRCA, so consequently, CS therapy was started. CS plus cyclophosphamide (CY) is also an effective therapy with a response rate of 87% (17), and we considered this as a secondary option if CS therapy proved ineffective. Successful re-treatment with ESAs in patients with detectable antibodies has been described, but this is associated with a high risk of exacerbation and is generally not recommended (8, 18). Hypoxia-inducible factor-prolyl hydroxylase inhibitors (HIF-PHIs) have been used to treat renal anemia. HIF-PHIs promote erythropoiesis primarily through increased endogenous EPO production and modulation of iron metabolism (19). Recently, antibody-mediated PRCA treated with roxadustat has been reported (20, 21). In one case, roxadustat was administered as re-treatment for renal anemia after immunosuppressive therapy (20). In another case, roxadustat was administered instead of immunosuppressive

Table 2. Antibody-mediated PRCA Cases Induced by CERA or Darbepoetin Alfa.

Case	Type of ESA	Age, gender	Diagnosis	Route of administration	Time to onset from start of ESA	Type of Ab	Immunosuppressive therapy	ESA retreatment	Outcome	Reference
1	CERA	42 y.o. male	Chronic glomerulonephritis HD	Not described	Several weeks	Not detected	CyA	Epoetin beta	Recovery after 4 weeks	5
2	CERA	44 y.o. male	Chronic pyelonephritis CAPD	S.C.	4 years	Anti-CERA antibody and anti-epoetin beta antibody	CyA+PSL	CERA (I.V.)	Recovery after 6 weeks. Both antibodies were undetectable after 20 weeks.	6
3	CERA	69 y.o. male	IgA nephropathy CKD stage 4	S.C.	7 months	Anti-CERA antibody and anti-EPO antibody	CyA → PSL	None	Recovery after 24 days. Both antibodies were undetectable after 12 weeks.	our case
4	Darbepoetin alfa	63 y.o. female	DM nephropathy CKD stage 3	S.C.	10 months	Anti-darbepoetin alfa and anti-epoetin alfa	CY+PSL	None	Recovery after 6 months	12
5	Darbepoetin alfa	78 y.o. male	Hypertensive nephrosclerosis CKD stage 4	S.C.	10 months	Anti-darbepoetin alfa and anti-epoetin alfa	CY+PSL	None	Recovery after 4 weeks	13
6	Darbepoetin alfa	76 y.o. male	Renovascular disease CKD stage 4	S.C.	14 months	Anti-EPO antibody	None	None	Not described	14
7	Darbepoetin alfa	58 y.o. male	Chronic glomerulonephritis HD	S.C.	21 months	Anti-EPO antibody	PSL → IVIG → CyA → PSL+CY	None	No response	15
8	Darbepoetin alfa	76 y.o. male	DM and hypertensive nephropathy CKD	S.C.	2 months	Not examined	PSL	None	Recovery after 2 months	16

PRCA: pure red cell aplasia, CERA: continuous erythropoietin receptor activator, ESA: erythropoiesis-stimulating agent, y.o.: year old, HD: hemodialysis, CAPD: continuous ambulatory peritoneal dialysis, IgA: immunoglobulin A, CKD: chronic kidney disease, DM: diabetes mellitus, S.C.: subcutaneous, EPO: erythropoietin, CyA: cyclosporine, CY: cyclophosphamide, PSL: prednisolone, I.V.: intravenous, IVIG: intravenous immunoglobulin, Ab: antibody

therapy, which improved PRCA (21). It is speculated that endogenous EPO upregulated by roxadustat helped neutralize anti-EPO antibodies but not boost the formation of antibodies to EPO (21). HIF-PHIs may thus be a safe option for treating renal anemia in patients with antibody-mediated PRCA.

Although the incidence of PRCA is decreasing, as described above, it remains an important complication in patients with CKD using ESAs. The reticulocyte count helps in the early diagnosis of PRCA during ESA treatment in patients with CKD. Furthermore, in PRCA not related to EPO antibodies, serum EPO levels increase as a result of the reduced consumption of EPO by erythroblasts, whereas in PRCA caused by neutralizing antibodies to EPO, serum EPO levels are usually extremely low (9). Anemia with both reticulocytopenia and low serum EPO levels indicates antibody-mediated PRCA in patients treated with ESAs. Clinicians should recognize that CERA, like other ESAs, induces antibody-mediated PRCA, and PRCA should be considered in the differential diagnosis of ESA-refractory anemia in patients with CKD.

The authors state that they have no Conflict of Interest (COI).

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